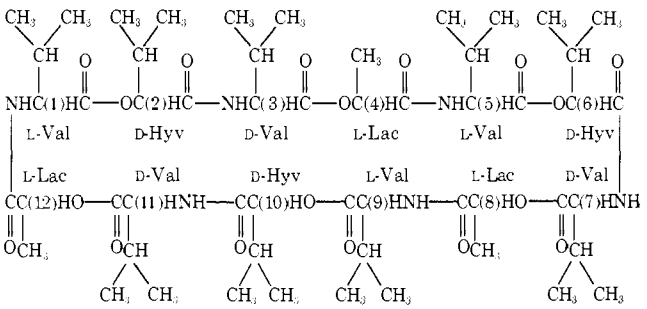
Conformation of Valinomycin in a Triclinic Crystal Form

Isabella L. Karle

Contribution from the Laboratory for the Structure of Matter, Naval Research Laboratory, Washington, D.C. 20375. *Received January 20, 1975*

Abstract: Uncomplexed valinomycin, (-L-Val-D-Hyv-D-Val-L-Lac-)$_3$, $C_{54}H_{90}N_6O_{18}$, grown from octane or acetone solution crystallizes in the triclinic space group $P1$ with two crystallographically unrelated molecules in the cell. The two independent molecules have the same conformation with four intramolecular hydrogen bonds of the 4 → 1 type (average NH···O = 2.95 Å) and two possible intramolecular hydrogen bonds of the 5 → 1 type involving ester carbonyl oxygens (average NH···O = 3.05 Å, H···O = 2.3 Å). Each molecule contains an approximate center of symmetry with the atoms in residues i and $i + 6$ related by the center, except for the differences in the chemical compositions of the side chains of Hyv and Lac. The gross shape of the molecule is that of a short, flattened oval tube with parallel ridges on the outer surface containing the hydrocarbon side chains and an interior surface lined with polar groups. The cell dimensions are $a = 22.285 \pm 0.015$ Å, $b = 10.360 \pm 0.012$ Å, $c = 14.525 \pm 0.013$ Å; $\alpha = 90.06 \pm 0.06°$, $\beta = 105.25 \pm 0.06°$, and $\gamma = 93.31 \pm 0.06°$. The structure containing 156 C, N, and O independent atoms was solved by the symbolic addition procedure.

Valinomycin, a cyclic dodecadepsipeptide with the formula (and numbering sequence)



has been the subject of extensive research since it was shown to have the ability to act as a mobile carrier to facilitate ion transport across cell membranes.[1-3] It exhibits a selectivity for K^+ or Rb^+ in preference to Na^+. A number of investigations of valinomycin and its K^+ complex in solution employing proton and ^{13}C NMR spectroscopy,[4-8] conformational calculations,[9] and small-angle X-ray diffraction studies[10] as well as Raman spectroscopy on single crystals[11] have established that valinomycin adopts a minimum of three different conformations: (A) the conformation of uncomplexed valinomycin in nonpolar solvents; (B) the conformation of uncomplexed valinomycin in polar solvents; and (C) the conformation of the K^+ complex.

An X-ray diffraction analysis of a single crystal can have the virtue of providing exact dimensions for the various possible conformations. Determinations of the conformation in the crystal by X-ray diffraction analysis have been reported for the K^+ complex of valinomycin[12] and for the uncomplexed valinomycin[13] crystallized in space group $P2_1$. No structural information such as atomic coordinates, conformational angles, or molecular dimensions has been reported yet in the literature.[31] In this paper, the complete crystal and molecular structure of valinomycin will be described. The crystal used in this determination, grown from octane, is triclinic, space group $P1$, with two crystallographically unrelated molecules in the cell. The presence of two independent molecules in the triclinic cell required the location of 156 C, N, and O atoms. The earlier report on uncomplexed valinomycin concerned a crystal in space group $P2_1$, with one molecule in the asymmetric unit.[13]

Crystals of valinomycin grown from octane and some other solvents are highly twinned and mimic $P2_1$ symmetry. It was rather difficult to obtain a crystal which was predominantly untwinned. The nature of the twinning will be discussed.

Experimental Section

Crystalline valinomycin purchased from Sigma Chemical Co. was in the form of stout colorless prisms with a soft texture that were easily deformed. Examination under a polarizing microscope, as well as X-ray diffraction photographs, showed that the crystals were highly twinned, usually in a 1:1 ratio. The twinned crystals mimic the monoclinic space group $P2_1$. Recrystallization from octane resulted in similar crystals, also highly twinned. By manual inspection, one crystal was found which was predominantly untwinned. Precession and Weissenberg photographs of the crystal mounted on the b^* axis showed that the true space group is triclinic. Cell parameters were determined by a least-squares fit to the 2θ, ω, ψ, and ϕ values of 12 reflections centered on a four-circle automatic diffractometer. Intensity data were collected for the major twin by the θ–2θ scan technique with nickel-filtered copper radiation. The scan width was $2.0° + 2\theta(\alpha_2)° - 2\theta(\alpha_1)°$, the scan speed was 2°/min, and the background was read for 10 sec at either end of the scan. Three reflections were monitored after every 50 measurements. A total of 8480 reflections were measured in the range $0° < 2\theta < 110°$. At scattering angles of $2\theta > 110°$, very few diffracting planes had measurable intensity.

The ratio of major to minor twin is $I_M/I_m \sim 15/1$ with complete overlap only for the $hk0$ reflections. Subsequently, untwinned triclinic crystals were grown from acetone with the same cell parameters and diffraction pattern as those grown from octane. These crystals were too minute to be used for intensity measurements.

Crystal data for the triclinic form of valinomycin follow: mol formula $C_{54}H_{90}N_6O_{18}$; mol wt 1048.87; space group $P1$; $Z = 2$; $a = 22.285 \pm 0.015$ Å, $b = 10.360 \pm 0.012$ Å, $c = 14.525 \pm 0.013$ Å; $\alpha = 90.06 \pm 0.06°$, $\beta = 105.25 \pm 0.06°$, $\gamma = 93.31 \pm 0.06°$; $V = 3229.3$ Å^3; $D_{calcd} = 1.078$ g/cm^3; crystal size, 0.45 × 0.50 × 0.45 mm; $\mu = 7.19$ cm^{-1}; $\lambda = 1.54178$ Å; total number of reflections, 8480.

Some years ago cell parameters were measured from a partially

Table I. Origin Specifications and Other Assignments for Implementing the Sum of Angles Formula

	h	ϕ_h	Values for E map	$\lvert E_h \rvert$
Origin	748	π		3.38
	41$\bar{2}$	π		4.01
	40$\bar{1}$	π		2.82
	40$\bar{2}$	a	π	3.87
	60$\bar{2}$	b	π	4.59
	$\bar{1}$10	c	0	3.51
	405	d	$+3/4\pi$	4.14
	$\bar{4}4\bar{1}0$	e	$+\pi/4$	3.33
	$\bar{6}5\bar{9}$	f	$+\pi/4$	2.98

Table II. Fractional Coordinates[19] and Isotropic Thermal Factors

Molecule	Atom[a]	x	y	z	B	Molecule	Atom[a]	x	y	z	B
I	N_1	0.0351	0.1344	−0.1562	4.49	I	C_{11}'	−0.0281	−0.1731	−0.3163	4.66
I	$C_1\alpha$	0.0569	0.2579	−0.1114	4.36	I	O_{11}	−0.0308	−0.2790	−0.2781	5.68
I	C_1'	0.0623	0.2539	−0.0021	4.47	I	$C_{11}\beta$	−0.0989	−0.2576	−0.4746	5.95
I	O_1	0.0183	0.2146	0.0283	6.16	I	$C_{111}\gamma$	−0.0514	−0.3596	−0.4841	6.32
I	$C_1\beta$	0.0155	0.3641	−0.1571	4.92	I	$C_{112}\gamma$	−0.1369	−0.2114	−0.5773	8.29
I	$C_{11}\gamma$	0.0408	0.4965	−0.1048	7.47	I	O_{12}'	0.0078	−0.0692	−0.2727	4.30
I	$C_{12}\gamma$	0.0161	0.3769	−0.2611	7.56	I	$C_{12}\alpha$	0.0502	−0.1011	−0.1781	4.97
I	O_2'	0.1161	0.2987	0.0514	4.45	I	C_{12}'	0.0707	0.0321	−0.1294	4.90
I	$C_2\alpha$	0.1242	0.3110	0.1515	4.62	I	O_{12}	0.1203	0.0437	−0.0668	5.39
I	C_2'	0.1532	0.1966	0.2002	4.85	I	$C_{12}\beta$	0.1037	−0.1716	−0.1944	8.29
I	O_2	0.1663	0.1907	0.2917	5.22	II	N_1	0.4472	0.5613	0.1284	5.07
I	$C_2\beta$	0.1671	0.4381	0.1842	5.74	II	$C_1\alpha$	0.4234	0.6807	0.0788	4.88
I	$C_{21}\gamma$	0.1628	0.4793	0.2846	7.17	II	C_1'	0.4241	0.6721	−0.0237	5.67
I	$C_{22}\gamma$	0.2321	0.4195	0.1753	7.69	II	O_1	0.4683	0.6447	−0.0535	6.63
I	N_3	0.1657	0.0962	0.1495	5.10	II	$C_1\beta$	0.4705	0.7976	0.1301	5.51
I	$C_3\alpha$	0.1932	−0.0153	0.2017	6.11	II	$C_{11}\gamma$	0.4434	0.9240	0.0730	7.14
I	C_3'	0.1500	−0.0737	0.2497	5.21	II	$C_{12}\gamma$	0.4657	0.8143	0.2322	7.86
I	O_3	0.0929	−0.1073	0.2140	6.73	II	O_2'	0.3689	0.7015	−0.0804	5.30
I	$C_3\beta$	0.2120	−0.1114	0.1366	6.81	II	$C_2\alpha$	0.3633	0.7115	−0.1834	5.74
I	$C_{31}\gamma$	0.2368	−0.2253	0.1723	13.36	II	C_2'	0.3340	0.5915	−0.2318	5.23
I	$C_{32}\gamma$	0.2590	−0.0475	0.0810	8.99	II	O_2	0.3237	0.5804	−0.3206	6.18
I	O_4'	0.1752	−0.1051	0.3420	5.56	II	$C_2\beta$	0.3240	0.8394	−0.2124	7.52
I	$C_4\alpha$	0.1326	−0.1705	0.3946	6.85	II	$C_{21}\gamma$	0.3301	0.8780	−0.3100	8.07
I	C_4'	0.0898	−0.0768	0.4199	6.24	II	$C_{22}\gamma$	0.2600	0.8041	−0.2141	9.88
I	O_4	0.0420	−0.1272	0.4416	7.96	II	N_3	0.3234	0.4830	−0.1785	5.88
I	$C_4\beta$	0.1806	−0.2143	0.4937	8.89	II	$C_3\alpha$	0.2945	0.3683	−0.2351	6.31
I	N_5	0.0972	0.0505	0.4032	5.11	II	C_3'	0.3363	0.3170	−0.2839	5.55
I	$C_5\alpha$	0.0521	0.1410	0.4186	4.85	II	O_3	0.3930	0.3014	−0.2509	7.11
I	C_5'	0.0144	0.1796	0.3180	5.50	II	$C_3\beta$	0.2802	0.2529	−0.1662	9.07
I	O_5	0.0211	0.2827	0.2798	6.17	II	$*C_{31}\gamma$	0.2382	0.1605	−0.2110	8.13
I	$C_5\beta$	0.0903	0.2677	0.4763	7.08	II	$*C_{31}\gamma'$	0.3362	0.1848	−0.1099	6.03
I	$C_{51}\gamma$	0.0451	0.3611	0.4877	8.35	II	$C_{32}\gamma$	0.2299	0.3157	−0.1269	11.97
I	$C_{52}\gamma$	0.1281	0.2125	0.5724	9.18	II	O_4'	0.3116	0.2851	−0.3747	6.14
I	O_6'	−0.0296	0.0871	0.2758	4.63	II	$C_4\alpha$	0.3506	0.2259	−0.4323	6.63
I	$C_6\alpha$	−0.0705	0.1126	0.1859	4.18	II	C_4'	0.3951	0.3314	−0.4561	6.53
I	C_6'	−0.0846	−0.0128	0.1313	4.26	II	O_4	0.4364	0.2951	−0.4896	8.13
I	O_6	−0.1318	−0.0208	0.0577	5.31	II	$C_4\beta$	0.3051	0.1752	−0.5274	7.87
I	$C_6\beta$	−0.1273	0.1607	0.2065	6.64	II	N_5	0.3864	0.4600	−0.4385	5.15
I	$*C_{61}\gamma$	−0.1625	0.2297	0.1304	8.95	II	$C_5\alpha$	0.4333	0.5579	−0.4525	4.54
I	$*C_{61}\gamma'$	−0.1274	0.2944	0.2589	4.94	II	C_5'	0.4692	0.6128	−0.3528	5.11
I	$C_{62}\gamma$	−0.1643	0.0600	0.2544	9.62	II	O_5	0.4652	0.7120	−0.3149	5.85
I	N_7	−0.0530	−0.1151	0.1623	4.02	II	$C_5\beta$	0.3936	0.6729	−0.5124	6.79
I	$C_7\alpha$	−0.0739	−0.2401	0.1143	4.33	II	$C_{51}\gamma$	0.4403	0.7879	−0.5219	6.80
I	C_7'	−0.0724	−0.2268	0.0086	4.54	II	$C_{52}\gamma$	0.3579	0.6145	−0.6091	9.55
I	O_7	−0.0291	−0.1824	−0.0184	5.91	II	O_6'	0.5108	0.5268	−0.3099	4.79
I	$C_7\beta$	−0.0258	−0.3440	0.1644	5.99	II	$C_6\alpha$	0.5537	0.5639	−0.2196	4.13
I	$C_{71}\gamma$	−0.0486	−0.4793	0.1062	6.40	II	C_6'	0.5701	0.4421	−0.1623	3.96
I	$C_{72}\gamma$	−0.0320	−0.3664	0.2642	6.66	II	O_6	0.6149	0.4472	−0.0894	5.23
I	O_8'	−0.1261	−0.2839	−0.0466	4.42	II	$C_6\beta$	0.6163	0.6310	−0.2407	5.46
I	$C_8\alpha$	−0.1281	−0.2946	−0.1477	4.89	II	$*C_{61}\gamma$	0.6494	0.6943	−0.1645	7.74
I	C_8'	−0.1654	−0.1790	−0.1999	3.59	II	$*C_{61}\gamma'$	0.6087	0.7463	−0.2814	6.78
I	O_8	−0.1784	−0.1833	−0.2884	5.22	II	$C_{62}\gamma$	0.6453	0.5475	−0.2891	8.33
I	$C_8\beta$	−0.1655	−0.4234	−0.1862	6.83	II	N_7	0.5357	0.3343	−0.1949	4.19
I	N_9	−0.1767	−0.0826	−0.1509	4.68	II	$C_7\alpha$	0.5571	0.2128	−0.1465	3.96
I	$C_9\alpha$	−0.2042	0.0276	−0.2019	4.09	II	C_7'	0.5551	0.2235	−0.0434	3.75
I	C_9'	−0.1571	0.0952	−0.2485	4.71	II	O_7	0.5126	0.2568	−0.0138	5.59
I	O_9	−0.1043	0.1248	−0.2110	6.37	II	$C_7\beta$	0.5127	0.0969	−0.1946	4.83
I	$C_9\beta$	−0.2204	0.1204	−0.1334	6.42	II	$C_{71}\gamma$	0.5327	−0.0320	−0.1385	6.06
I	$C_{91}\gamma$	−0.2476	0.2483	−0.1837	8.00	II	$C_{72}\gamma$	0.5192	0.0753	−0.2982	6.37
I	$C_{92}\gamma$	−0.2709	0.0537	−0.0902	7.89	II	O_8'	0.6078	0.1830	0.0163	4.07
I	O_{10}'	−0.1823	0.1169	−0.3431	4.71	II	$C_8\alpha$	0.6125	0.1726	0.1160	4.50
I	$C_{10}\alpha$	−0.1411	0.1831	−0.3947	5.55	II	C_8'	0.6456	0.2953	0.1662	5.29
I	C_{10}'	−0.0991	0.0828	−0.4256	5.64	II	O_8	0.6628	0.2948	0.2575	5.13
I	O_{10}	−0.0586	0.1259	−0.4589	7.16	II	$C_8\beta$	0.6461	0.0533	0.1523	5.74
I	$C_{10}\beta$	−0.1817	0.2427	−0.4866	8.82	II	N_9	0.6605	0.3959	0.1190	4.19
I	$C_{101}\gamma$	−0.2060	0.3606	−0.4545	15.32	II	$C_9\alpha$	0.6885	0.5090	0.1704	4.56
I	$C_{102}\gamma$	−0.2295	0.1508	−0.5401	7.31	II	C_9'	0.6405	0.5678	0.2194	4.34
I	N_{11}	−0.1093	−0.0465	−0.4054	5.34	II	O_9	0.5883	0.5862	0.1798	6.83
I	$C_{11}\alpha$	−0.0637	−0.1386	−0.4185	4.47	II	$C_9\beta$	0.7045	0.6107	0.1008	5.43

Table II (*Continued*)

Molecule	Atom[a]	x	y	z	B
II	$C_{91}\gamma$	0.7294	0.7432	0.1547	7.12
II	$C_{92}\gamma$	0.7552	0.5595	0.0557	7.60
II	O_{10}'	0.6667	0.5978	0.3134	5.21
II	$C_{10}\alpha$	0.6253	0.6485	0.3659	5.48
II	C_{10}'	0.5836	0.5378	0.3915	5.57
II	O_{10}	0.5411	0.5764	0.4238	7.24
II	$C_{10}\beta$	0.6629	0.7244	0.4524	8.33
II	$C_{101}\gamma$	0.6967	0.8442	0.4340	15.50
II	$C_{102}\gamma$	0.7116	0.6445	0.5061	7.90
II	N_{11}	0.5952	0.4180	0.3751	4.90
II	$C_{11}\alpha$	0.5492	0.3115	0.3878	4.64
II	C_{11}'	0.5120	0.2704	0.2823	5.47
II	O_{11}	0.5167	0.1645	0.2470	5.96
II	$C_{11}\beta$	0.5857	0.1982	0.4420	6.31
II	$C_{111}\gamma$	0.5366	0.0841	0.4491	7.67
II	$C_{112}\gamma$	0.6237	0.2558	0.5462	8.99
II	O_{12}'	0.4740	0.3605	0.2409	4.72
II	$C_{12}\alpha$	0.4336	0.3204	0.1484	5.04
II	C_{12}'	0.4105	0.4487	0.1018	6.03
II	O_{12}	0.3634	0.4484	0.0380	6.17
II	$C_{12}\beta$	0.3804	0.2413	0.1664	6.83
		Standard Deviations			
	N	0.0008	0.0016	0.0011	
	O (ring)	0.0006	0.0012	0.0009	
	$C\alpha$	0.0009	0.0020	0.0015	
	C'	0.0010	0.0020	0.0015	
	O (carbonyl)	0.0007	0.0014	0.0011	
	$C\beta$	0.0012	0.0024	0.0018	
	$C\gamma$	0.0013	0.0027	0.0020	
	$*C\gamma$	0.0026	0.0054	0.0040	

[a] * represents disordered atoms.

twinned crystal for the triclinic form of valinomycin by Mathieson.[14] His values were $a = 10.4_4$ Å, $b = 14.47$ Å, $c = 22.2_2$ Å; $\alpha = 105.0°$, $\beta = 86.9°$, $\gamma = 90.4°$. The labels for the axes and angles are different, but the cell is equivalent to the one reported in this paper.

The intensity data were corrected for Lorentz and polarization factors, but not for absorption. Normalized structure factors $|E|$ were derived and the statistical averages are $\langle|E|\rangle = 0.809$ and $\langle||E|^2 - 1|\rangle = 0.877$. Corresponding theoretical values for centrosymmetric crystals are 0.798 and 0.968 and for noncentrosymmetric they are 0.886 and 0.736, respectively. The statistical averages for valinomycin can be interpreted to imply that the space group is noncentrosymmetric and that there are some pseudocentrosymmetric features in the structure. The crystal was treated as if it were in space group $P1$ with two independent molecules having 156 independent C, N, and O atoms to be located. The results of the structure determination confirmed this interpretation of the statistics.

The structure was solved by obtaining values for the phases directly from the measured intensities by the symbolic addition procedure as applied to noncentrosymmetric space groups.[15,16] The origin specification and the additional reflections to which symbols were assigned for the implementation of the sum of angles formula, $\langle\phi_{\mathbf{h}} \approx \phi_{\mathbf{k}} + \phi_{\mathbf{h}-\mathbf{k}}\rangle_{k_r}$, are shown in Table I. (Initially the origin was assigned with three zero-phase values. Later a new origin was chosen for a better placement of the molecules in the cell and the phases shown in Table I are consistent with the coordinates in Table II). There were some indications of the values of *a*, *b*, and *c* during the phase determination. The enantiomorph specification is made by choosing a (+) or (−) value for a phase significantly different than 0 or π. Since the experimental values for statistical averages fall between the values expected for centric and acentric space groups, it was prudent to assume that the phase values for symbols *d*, *e*, and *f* would be near 0 or π. Hence a number of possibilities were examined with values of $\pm\pi/4$ and $\pm 3/4\pi$. The values listed in Table I led to an *E* map from which 75 atomic positions could be selected as a partial structure for further development. The atomic positions were used to compute new phases for the tangent formula[17] in the partial structure development[18] which led to the complete structure.

A full-matrix least-squares refinement was carried out to convergence on the coordinates of the 156 atoms with isotropic thermal factors. The function minimized was $\Sigma w_F(|F_o| - |F_c|)^2$ where $w_F = 1/\sigma^2_F$. The quantity $\sigma^2_F = (A/4Lp)(\sigma^2_I/I)$ where A is the attenuator factor, LP is the Lorentz-polarization correction, and $\sigma^2_I = \sigma^2_P + \sigma^2_{B_1} + \sigma^2_{B_2}$ where P is the peak count, B_1 and B_2 are the scaled background counts, and σ^2_N for any count N is given by $\sigma^2_N = N + (0.015)^2N^2$. Of the 624 variables, plus one more for the scale, 241 were refined in each pass. Three passes constituted one cycle of refinement. In each pass, the parameters which were varied were those of adjacent atoms and included some overlap from the previous pass. Only those data with $|F_o| > 5.0$, 5456 in all, were used in the refinement owing to the limitations of computer size and cost. No hydrogen atoms were included in the calculations. The coordinates reported in Table II are those at an *R* factor of 12.0% where $R = \Sigma||F_o| - |F_c||/\Sigma|F_o|$.

In the course of refinement, the thermal factors for one terminal methyl carbon atom in residue 3 in molecule I and residues 3 and 6 in molecule II were unreasonably high. An examination of a difference map showed additional positions for these atoms. Based on the values of the thermal factors for these atoms at this stage of refinement and the difference map, it appears that atoms $IC_6\gamma$, $IIC_3\gamma$, and $IIC_6\gamma$ are disordered between two equivalent positions. These atoms are marked with an asterisk in Tables II and IV. (No further refinement is planned at this time. See paragraph at end of paper regarding supplementary material.)

Fractional coordinates for the 156 nonhydrogen atoms in the two molecules are listed in Table II, bond lengths and angles for the peptide chain are shown in Table III, and the conformational angles are shown in Table IV.

Results

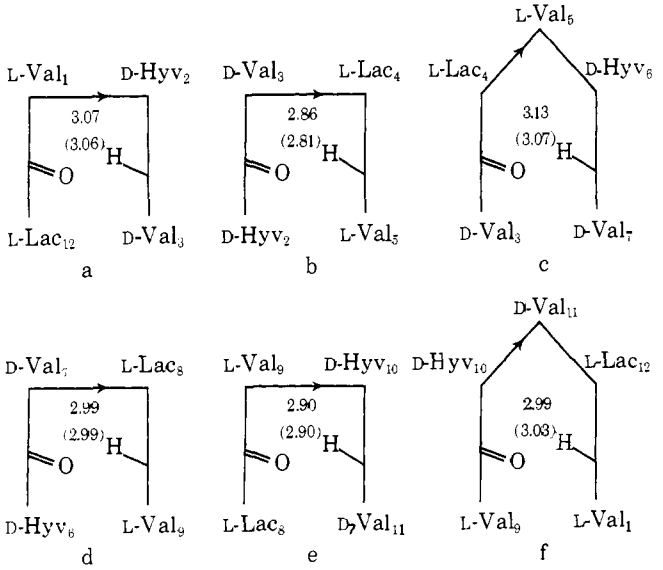
Dodecadepsipeptide Ring. The two independent molecules in the cell have essentially the same conformation. Conformational angles,[19] listed in Table IV, are very similar in magnitude and sign for the two molecules. Each polypeptide ring consisting of 36 atoms has three loops up and three loops down around a flattened oval. The stereodiagrams in Figure 1 show two views of the molecule. A possible threefold symmetry as suggested by the threefold repetition in the molecular formula is not maintained in this crystalline form derived from octane or acetone solutions. Rather, there is an approximate center of symmetry in each molecule which relates residues *i* with residues $i + 6$ quite well, except, of course, for the different side chains in L-Lac and D-Hyv. The pseudocenter in each molecule is manifested by conformational angles for residues *i* and $i + 6$ having nearly the same magnitudes and opposite signs. The residues in valinomycin alternate between amino acid and hydroxy acid groupings, $-NH(C^{\alpha}HR)(CO)-$ and $-O(C^{\alpha}HR)(CO)-$, respectively. Each of the residues is in the trans conformation and is essentially planar as indicated by the ω_i values which are all near 180°. At the present stage of refinement, the deviations from 180° for the ω_i values are not particularly meaningful. A plot of the ϕ,ψ angles is shown in Figure 2.[20]

A comparison of the conformation of the valinomycin molecules determined in this study with that reported for valinomycin crystallizing in space group $P2_1$[13] is made in Figure 3 where the upper diagram depicts a molecule from the present study in an orientation similar to the diagram published by Duax et al.[13] Atomic coordinates and molecular parameters have not been published for the latter molecule. It appears that there is a close similarity between the two.

There are only six NH groups available for hydrogen bonding and six possible intramolecular NH···O=C bonds between the following residues are formed.

Table III. Bond Lengths (Å) and Angles (deg)

Bonds	*i* 1	2	3	4	5	6	7	8	9	10	11	12	Av	
Molecule I														
$C_{i-1}'-N_i$	1.36		1.36		1.35		1.32		1.30		1.39		1.35	
$C_{i-1}'-O_i'$		1.30		1.36		1.35		1.36		1.36		1.35	1.35	
$N_i-C_i^\alpha$	1.43		1.46		1.47		1.46		1.44		1.48		1.46	
$O_i'-C_i^\alpha$		1.42		1.50		1.42		1.46		1.47		1.50	1.46	
$C_i^\alpha-C_i'$	1.56	1.47	1.44	1.51	1.55	1.50	1.55	1.58	1.53	1.58	1.54	1.54	1.53	
$C_i'-O_i$	1.23	1.29	1.27	1.27	1.22	1.29	1.20	1.24	1.18	1.20	1.24	1.23	1.24	
Angles														
$C_{i-1}'N_iC_i^\alpha$	119		118		121		119		118		118		119	
$C_{i-1}'O_i'C_i^\alpha$		119		117		119		114		117		113	117	
$N_iC_i^\alpha C_i'$	111		109		106		108		109		105		108	
$O_i'C_i^\alpha C_i'$		110		111		107		107		110		104	108	
$C_i^\alpha C_i'O_i$	121	120	128	116	125	118	126	116	126	117	126	119	125	*i* odd
													118	*i* even
$C_i^\alpha C_i'N_{i+1}$		121		120		121		120		117		120	120	
$C_i^\alpha C_i'O_{i+1}'$	114		115		112		108		112		110		112	
$O_iC_i'N_{i+1}$		119		123		121		124		126		121	122	
$O_iC_i'O_{i+1}'$	124		117		122		126		121		124		122	
	L-Val	D-Hyv	D-Val	L-Lac	L-Val	D-Hyv	D-Val	L-Lac	L-Val	D-Hyv	D-Val	L-Lac		
Molecule II														
Bonds														
$C_{i-1}'-N_i$	1.38		1.41		1.39		1.33		1.32		1.32		1.36	
$C_{i-1}'-O_i'$		1.34		1.32		1.35		1.35		1.36		1.33	1.34	
$N_i-C_i^\alpha$	1.49		1.46		1.47		1.48		1.41		1.51		1.47	
$O_i'-C_i^\alpha$		1.47		1.51		1.44		1.43		1.46		1.45	1.46	
$C_i^\alpha-C_i'$	1.50	1.46	1.43	1.53	1.55	1.52	1.51	1.52	1.58	1.54	1.58	1.55	1.52	
$C_i'-O_i$	1.22	1.25	1.25	1.23	1.18	1.25	1.21	1.28	1.18	1.25	1.23	1.20	1.23	
Angles														
$C_{i-1}'N_iC_i^\alpha$	117		115		118		117		119		118		117	
$C_{i-1}'O_i'C_i^\alpha$		118		120		118		120		116		113	117	
$N_iC_i^\alpha C_i'$	110		111		107		108		109		104		108	
$O_i'C_i^\alpha C_i'$		110		109		108		109		110		104	108	
$C_i^\alpha C_i'O_i$	126	120	127	117	130	119	127	117	125	113	122	120	126	*i* odd
													118	*i* even
$C_i^\alpha C_i'N_{i+1}$		120		119		117		122		118		119	119	
$C_i^\alpha C_i'O_{i+1}'$	110		116		108		111		112		112		111	
$O_iC_i'N_{i+1}$		119		125		124		120		128		120	123	
$O_iC_i'O_{i+1}'$	124		117		121		121		123		126		122	



The N···O distances in the possible NH···O=C hydrogen bond are shown for both molecules with those for molecule II in parentheses. These values cover a range of 2.81–3.13 Å. Intramolecular hydrogen bond distances of the same order of magnitude have been observed in other cyclic polypeptides.[21,22]

Four of the intramolecular hydrogen bonds are of the familiar 4 → 1 type,[23] first observed in cyclohexaglycyl.[24] Intramolecular hydrogen bonds of the type 4 → 1 exist in two favored conformations, called I and II by Venkatachalam.[23] Type I has been observed in cyclo hexaglycyl,[24] cyclo-4-Gly-2-D-Ala,[22] alkali metal complexes of antamanide,[21] the C-terminal tetrapeptide of oxytocin,[25] and *p*-bromocarboxy-Gly-L-Pro-L-Leu-Gly,[26] for example, where residues 2 and 3 have been (Gly,Gly), (D-Ala,D-Ala), (L-Ala,L-Phe), (L-Phe,L-Phe), and (L-Pro,L-Leu).

Type II has occurred in ferrichrome A,[27] for example, where residues 2 and 3 are L-Ser and Gly. The 4 → 1 hydrogen bonds in valinomycin, a, b, d, and e, are similar to type II bonds with the exception that the NH in residue 3 has been replaced with O (see type II_O). The ϕ_2,ψ_2 and ϕ_3,ψ_3 angles for ferrichrome A are −57,+132° and +82,−1°, respectively, while the value for the equivalent angles in valinomycin for hydrogen bond a are −63,+129° and +96,−3° and there are very similar values for hydrogen bonds b, d, and e in both valinomycin molecules; see Table IV. In Figure 2, the pairs involved in these bends are (V1, H2), (V3, L4), (V7, L8), and (V9, H10). It appears

Table IV. Conformational Angles [19] in Valinomycin[a]

i	Molecule	L-Val 1	D-Hyv 2	D-Val 3	L-Lac 4	L-Val 5	D-Hyv 6	D-Val 7	L-Lac 8	L-Val 9	D-Hyv 10	D-Val 11	L-Lac 12
ϕ_i	I	−63	96	63	−74	−108	146	60	−98	−67	82	108	−164
	II	−68	99	68	−75	−110	150	63	−96	−65	77	104	−162
ψ_i	I	129	−3	−134	−6	78	−11	−135	14	130	3	−69	23
	II	130	−8	−134	−11	78	−12	−134	6	132	8	−71	27
ω_i	I	174	−179	−178	174	176	172	−172	173	179	−170	−172	−178
	II	174	−179	−178	174	174	172	−174	177	−178	−172	−173	178
χ_i^1	I	179	164	−178		177	160	−178		177	76	−177	
	II	179	166	161		175	164	−176		174	66	−177	
χ_i^2	I	−62	−68	57		−62	−64, +67 *	68		−64	−49	62	
	II	−67	−75	66, −74 *		−64	−60, +70 *	68		−65	−51	63	

[a] An asterisk indicates one CH_3 group in each of these side chains is disordered among two possible positions.

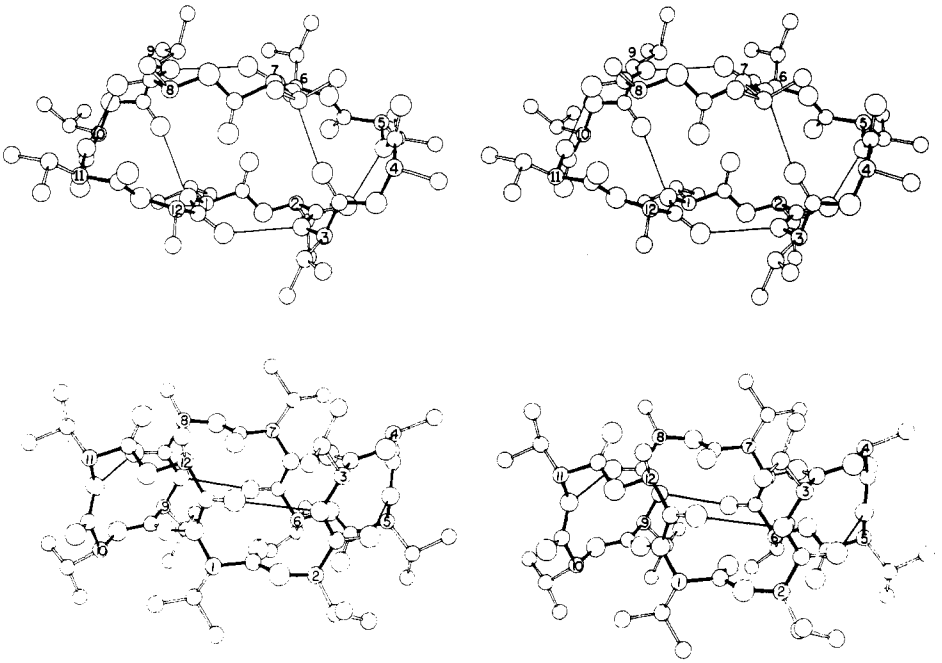
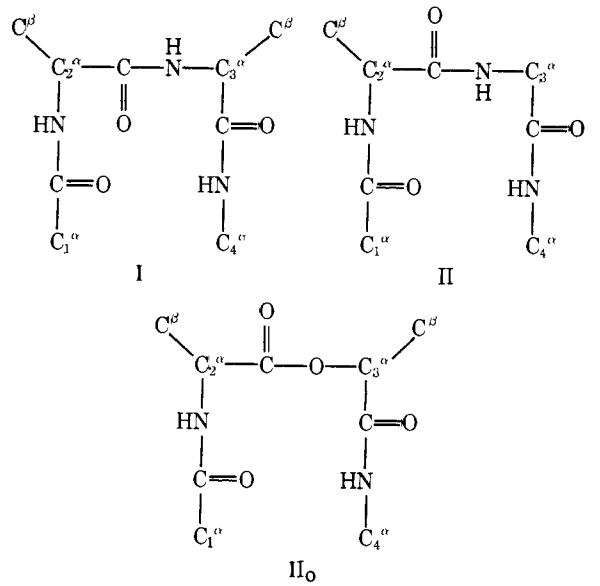


Figure 1. Stereodiagrams drawn with the experimentally determined coordinates for valinomycin crystallized from octane (or acetone). The two independent molecules in the cell have essentially the same conformation; hence, only one molecule is illustrated in the figure. The bonds in the 36-membered ring are darkened, the numbers refer to the C^α atoms in the 12 residues, and only one of two possible conformations for some of the disordered side chains is shown. Light lines depict the six possible NH···O=C intramolecular hydrogen bonds. The two views are at 90° to each other. The diagrams were drawn by a computer program (C. K. Johnson, Oak Ridge National Laboratory).



I II II_O

that the conformational angles for the type II 4- → 1- hydrogen bond are well established, regardless of whether the linkage between $C_2^\alpha \cdots C_3^\alpha$ is an amide or an ester. The exchange of -O- for -NH does not affect noticeably the spacings or conformation in the bends associated with the 4 → 1 type bond. The observed conformational values are near those calculated by Venkatachalam[23] for a three-linked peptide unit.

The 5 → 1 hydrogen bonds, c and f, have been observed only in valinomycin so far. They are characterized by points (L4, V5, H6) and (H10, V11, L12) in the conformational map, Figure 2. Formation of the 5 → 1 type of bond is associated with the flattened oval shape of the molecule as a whole, Figure 1. The N_1H moiety instead of binding to O_{10}, as would be the case if the molecule were to assume threefold symmetry, reaches across the ring to the ester carbonyl oxygen O_9. Similarly N_7H binds to O_3 instead of O_4. Owing to the threefold repetition of residues, the same conformation for the molecule is obtained if N_3 and N_9H or N_5H and $N_{11}H$ were to form 5 → 1 type hydrogen bonds instead of the 4 → 1 type. The flattening of the ring causes the car-

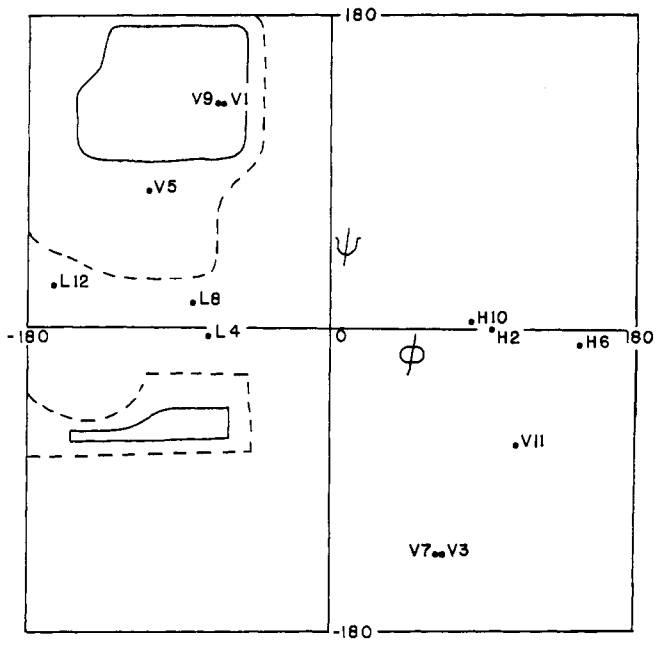


Figure 2. Conformational map for the experimental ϕ,ψ values in the 12 residues in molecule I of valinomycin. The dashed lines enclose the areas representing low-energy areas while the solid lines enclose the lowest energy areas as calculated by Ramachandran and Sasisekharan[20] for a pair of planar L-peptide units when C^β is present. For the case of D-peptide groups, the allowed areas are obtained by inverting about the point (0°,0°).

bonyl oxygen atoms, O_4 and O_{10}, to be exposed on the exterior surface of the molecule.

The 5 → 1 hydrogen bonds in valinomycin are relatively weaker than the 4 → 1 type. Not only is the average value for the N···O distance larger, 3.05 Å as compared to 2.95 Å, but also the NH···O angle deviates greatly from 180° in the 5 → 1 bonds. If the proton on the N atom is assumed to be in the plane of the peptide group with an N–H distance of 1.05 Å, then the NH···O angles are 120–125° in the 5 → 1 bonds and the H···O distances are near 2.32 Å. Since the sum of the van der Waals' radii for H and O is taken to be 2.6 Å,[28] a value of 2.32 Å for the H···O distance represents a very weak attraction. Strong hydrogen bonds have H···O separations in the range of 1.6–2.1 Å, as shown in neutron diffraction experiments of amino acids and dipeptides.[29]

Individual values for bond lengths and angles, Table III, have relatively high standard deviations, 0.026 Å and 2.2°, respectively, owing to the termination of the least-squares refinement at the isotropic stage and the omission of 180 hydrogen atoms; however, the average of many values for each type of bond and angle is meaningful. It can be seen from a comparison of the averaged values in Table III for the amide linkages and the ester linkages that the bond lengths are the same and that angle $C_{i-1}'N_iC_i^\alpha$ is essentially the same as angle $C_{i-1}'O_iC_i^\alpha$. There is a pronounced difference, however, in the angles about the C_i' atoms in the amide and ester groups

A similar effect had been observed in a cyclic tetradepsipeptide.[30]

Side Chains. The side chains are all directed to the exterior surface of the molecule. Nine of the C^α atoms have isopropyl moieties as side chains while the three lactate groups have only a CH_3 group. These three residues (i = 4, 8, and 12) all occur at the top rim of the cavity as displayed in Figure 1. Thus the top rim of the cavity is less hydrophobic than the bottom rim since the top contains three methyl groups and three isopropyl groups while the bottom is rimmed with six isopropyl groups. In each of the six valyl residues, the conformation of the isopropyl groups is such that one C^γ is trans to the N atom ($\chi_i^1 \sim 180°$) and the hydrogen on C^β is trans to the hydrogen on C^α, except in Val_3, molecule II, where one of the C^γ atoms is disordered among two positions.

The conformations of the isopropyl side chains are different in each of the three hydroxyisovaleryl moieties (i = 2, 6, and 10). In residue 6, one of the C^γ atoms is disordered among two positions (for both molecules I and II). In residue 2, the hydrogen on C^β is trans to C′ while in residue 10, the hydrogen on C^β is trans to O′. It is obvious in residue 10 that if HC^β were trans to HC^α, then the carbonyl oxygen O_{10}, which protrudes from the outer surface of the molecule would be shielded by a hydrophobic group. These results are in general agreement with those of proton resource studies of the K^+ complex in solution.[7]

The aliphatic groups are stacked in pairs, i.e., side chain from residue 3 over that of residue 2, 4 over 5, 7 over 6, etc. (see Figure 1), so that the outer surface of a space-filling model of the molecule has a corrugated appearance with vertical ridges. The ridges are hydrophobic while the troughs between the ridges contain some of the oxygen atoms, including the carbonyl oxygens O_4 and O_{10}. The inner surface of the molecule is highly polar, being lined with oxygen atoms.

Packing. The packing of the molecules in the unit cell is depicted in Figure 4. Molecule I is shown at the corners of the cell while molecule II is near the middle. Although each molecule contains an approximate center of symmetry, the packing in the crystal is acentric. Molecules I and II are approximately related by a noncrystallographic twofold screw axis. An exact relationship is, of course, not possible since angle γ is 93.3°. The deviation from a twofold screw relationship is manifested by the intensities of reflections hkl being unequal to those of hkl and by large magnitudes for $|F_{010}|$ and $|F_{030}|$.

The forces between molecules are all van der Waal's attractions. The closest approaches, $O_{10}\cdots C_5^{\alpha'}$, $O_{10}\cdots C_{51}^{\gamma'}$, $O_4\cdots C_{11}^{\alpha'}$, and $O_4\cdots C_{111}^{\gamma'}$ at 3.39, 3.49, 3.49, and 3.45 Å, respectively, are between neighboring molecules I···I′ and II···II′, along the c direction. There are 15 C···O intermolecular distances between 3.4 and 3.65 Å. For C···C nearest intermolecular approaches, there are three in the range 3.5–3.6 Å and 14 in the range 3.7–3.8 Å. The closest C···C approach is between $C_{102}^{\gamma}\cdots C_{101}^{\gamma'}$ at 3.46 Å between neighboring molecules I and II′ along the cell diagonal.

Twinning. Valinomycin grown from octane has a very high propensity for twinning. The twinned crystals are in the form of prisms elongated along the b axis. They yield precession photographs which mimic $P2_1$ symmetry. The a^*b^* net shows mm symmetry with the 010 and 030 reflections very weak. Careful examination shows that the net is doubled with a common a^* axis. Rotation of the dial by +105°, equivalent to angle β, results in a photograph of the b^*c^* net, apparent mm symmetry; in addition, rotation of the dial axis by −99.5° yields another net with apparent mm symmetry and axial dimensions of b' = 10.35 and c' = 14.5. This latter plane does not occur in the crystal used for the experimental data on which the structural results in this paper were based. A Weissenberg photograph of the $h0l$ reflections in the twinned crystals show several reflections at

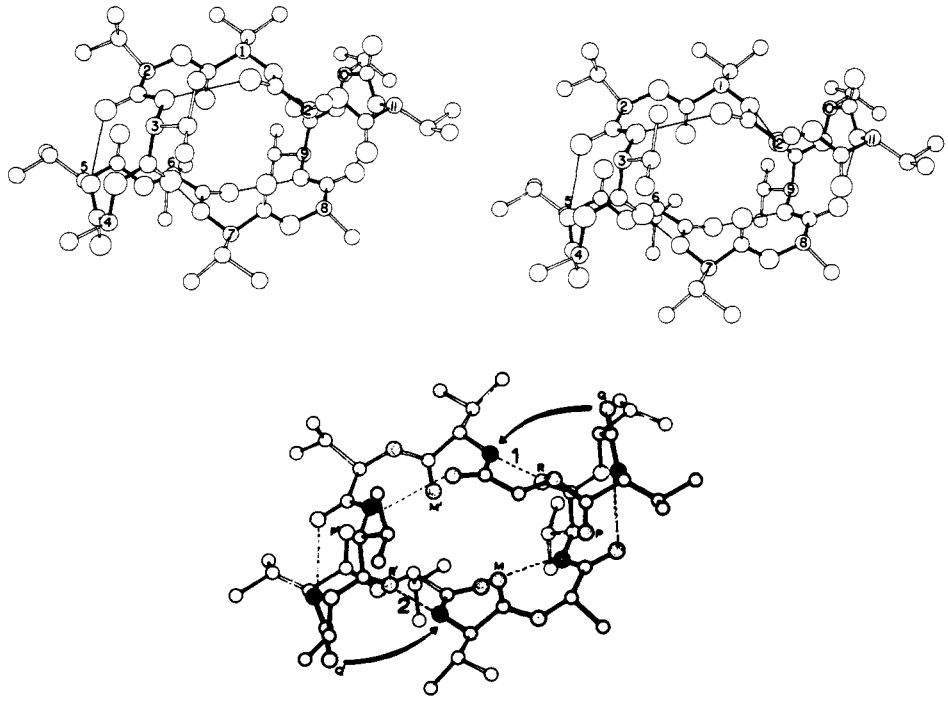


Figure 3. Stereodiagram of valinomycin crystallizing in space group $P1$ (upper figure) compared to a diagram of valinomycin in space group $P2_1$ published by Duax et al.[13] (lower figure).

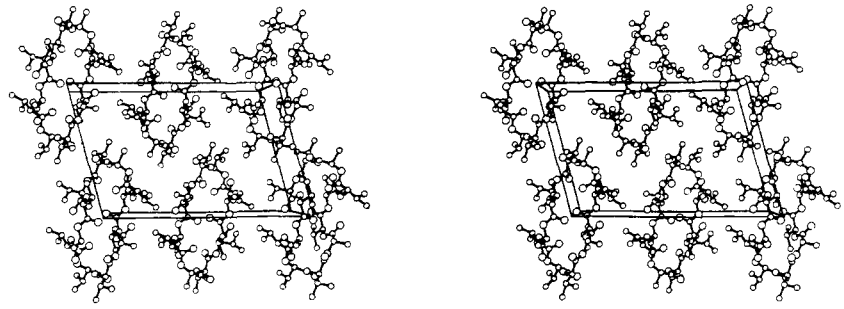


Figure 4. Stereodiagram of the packing in the triclinic cell. Molecule I and its translational equivalents are shown at the corners while molecule II and a translational equivalent are near the middle. The axes are directed so that a is horizontal, c points upward, and b points into the page.

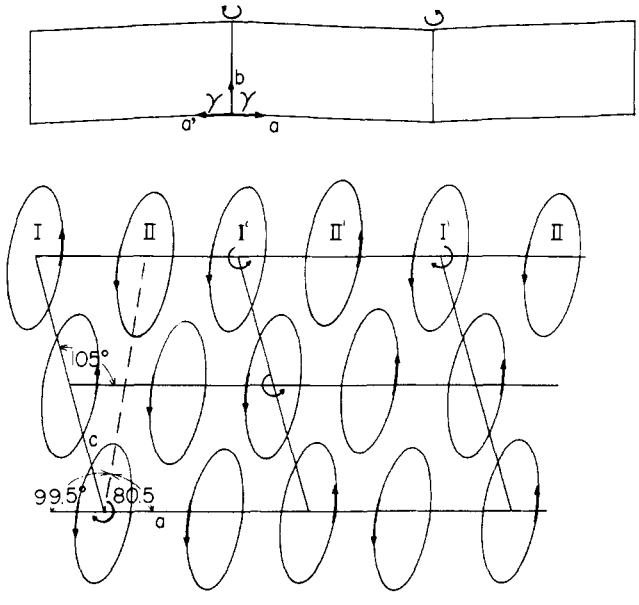


Figure 5. Possible twinning mechanism by random, multiple, twofold rotations about the b axis.

high scattering angles that indicate a doubled value for the a axis.

A possible twinning scheme consistent with the diffraction data is illustrated in Figure 5. Mimetic twinning occurs by multiple twofold rotations of the cells or individual molecules about axes parallel to the b axis. The valinomycin molecule occupies essentially the same space in the cell in both orientations as illustrated schematically in the lower diagram of Figure 5. A net in the twinned crystal can have the dimensions $2a$, b', $2c'$ (where $a = 22.3$, $b' = 10.35$, $c' = 14.5$ Å) and $\beta' = 99.5°$ with $\alpha = \gamma = 90°$. It is interesting to note the close correspondence between the latter values and those reported for valinomycin[13] in space group $P2_1$: $a = 23.14$, $b = 10.356$, $c = 14.526$ Å, and $\beta = 99.57°$.

Complexation. Speculations upon the mechanism of complexation and ion transport will be deferred until the conformation of crystalline uncomplexed valinomycin grown from a polar solvent is established. Crystals from dimethyl sulfoxide have the orthorhombic space group $P2_12_12_1$ with cell dimensions $a = 16.406 \pm 0.006$ Å, $b = 25.723 \pm 0.006$ Å, and $c = 18.712 \pm 0.005$ Å. The volume available for each molecule, assuming $Z = 4$, is 1974 Å^3, a value considerably larger than the volume per valinomycin molecule in the crystal from octane, 1615 Å^3. Hence, it is very probable

that some of the polar solvent is cocrystallized with the valinomycin in the orthorhombic cell and that the conformation of the molecule is different than that reported in this paper.

Acknowledgment. This project was supported in part by the Office of Naval Research Project Order P04-0095 and in part by the NIAMDD of the National Institutes of Health.

Supplementary Material Available. Tables of observed and calculated structure factors will appear following these pages in the microfilm edition of this volume of the journal. Photocopies of the supplementary material from this paper only or microfiche (105 × 148 mm, 24× reduction, negatives) containing all of the supplementary material for the papers in this issue may be obtained from the Journals Department, American Chemical Society, 1155 16th St., N.W., Washington, D.C. 20036. Remit check or money order for $4.50 for photocopy or $2.50 for microfiche, referring to code number JACS-75-4379.

References and Notes

(1) P. Mueller and D. O. Rudin, *Biochem. Biophys. Res. Commun.*, **26**, 398 (1967).
(2) D. C. Tosteson, P. Cook, T. E. Andreoli, and M. Tieffenberg, *J. Gen. Physiol.*, **50**, 2513 (1967).
(3) E. J. Harris and B. C. Pressman, *Nature (London)*, **216**, 918 (1967).
(4) V. T. Ivanov, I. A. Laine, N. D. Abdulaev, L. B. Senyavina, E. M. Popov, Yu. A. Ovchinnikov, and M. M. Shemyakin, *Biochem. Biophys. Res. Commun.*, **34**, 803 (1968).
(5) C. C. Haynes, A. Kowalsky, and B. C. Pressman, *J. Biol. Chem.*, **244**, 502 (1969).
(6) M. Ohnishi and D. W. Urry, *Biochem. Biophys. Res. Commun.*, **36**, 194 (1969).
(7) M. Ohnishi and D. W. Urry, *Science*, **168**, 1091 (1970).
(8) D. J. Patel, *Biochemistry*, **12**, 496 (1973).
(9) D. J. Patel and A. E. Tonelli, *Biochemistry*, **12**, 486 (1973).
(10) W. R. Krigbaum, F. R. Kuegler, and H. Oeischlaeger, *Biochemistry*, **11**, 4548 (1972).
(11) K. J. Rothschild, I. M. Asher, E. Anastassakis, and H. E. Stanley, *Science*, **182**, 384 (1973).
(12) M. Pinkerton, L. K. Steinrauf, and P. Dawkins, *Biochem. Biophys. Res. Commun.*, **35**, 512 (1969).
(13) W. L. Duax, H. Hauptman, C. M. Weeks, and D. A. Norton, *Science*, **176**, 911 (1972).
(14) A. McL. Mathieson, *Acta Crystallogr.*, **12**, 478 (1959).
(15) I. L. Karle and J. Karle, *Acta Crystallogr.*, **17**, 835 (1964).
(16) J. Karle and I. L. Karle, *Acta Crystallogr.*, **21**, 849 (1966).
(17) J. Karle and H. Hauptman, *Acta Crystallogr.*, **9**, 635 (1956).
(18) J. Karle, *Acta Crystallogr., Sect. B*, **24**, 182 (1968).
(19) The convention followed for labeling atoms and conformational angles is that proposed by the IUPAC–IUB Commission on Biochemical Nomenclature, *Biochemistry*, **9**, 3471 (1970). In the fully extended chain $\phi_l = \psi_l = \omega_l = 180°$.
(20) G. N. Ramachandran and V. Sasisekharan, *Adv. Protein Chem.*, **23**, 327 (1968).
(21) I. L. Karle, *J. Am. Chem. Soc.*, **96**, 4000 (1974).
(22) I. L. Karle, J. W. Gibson, and J. Karle, *J. Am. Chem. Soc.*, **92**, 3755 (1970).
(23) C. M. Venkatachalam, *Biopolymers*, **6**, 1425 (1968).
(24) I. L. Karle and J. Karle, *Acta Crystallogr.*, **16**, 969 (1963).
(25) A. D. Rudko, F. M. Lovell, and B. W. Low, *Nature (London), New Biol.*, **232**, 18 (1971).
(26) T. Ueki, T. Ashida, M. Kakudo, Y. Sasada, and Y. Katsube, *Acta Crystallogr., Sect. B*, **25**, 1840 (1969).
(27) A. Zalkin, J. D. Forrester, and D. H. Templeton, *J. Am. Chem. Soc.*, **88**, 1810 (1966).
(28) L. Pauling, "The Nature of the Chemical Bond", Cornell University Press Ithaca, N.Y., 1960, p 260.
(29) See, e.g., T. F. Koetzle, W. C. Hamilton, and R. Parthasarathy, *Acta Crystallogr., Sect. B*, **28**, 2083 (1972).
(30) J. Konnert and I. L. Karle, *J. Am. Chem. Soc.*, **91**, 4888 (1969).
(31) Note Added in Proof. A reinvestigation of the K^+ complex of valinomycin with complete structural data has been published by K. Neupert-Lares and M. Dobler, *Helv. Chim. Acta*, **58**, 432 (1975).

4′-Substituted Nucleosides. I. Synthesis of 4′-Methoxyuridine and Related Compounds

Julien P. H. Verheyden and John G. Moffatt*

Contribution No. 113 from the Institute of Molecular Biology, Syntex Research, Palo Alto, California 94304. *Received February 5, 1975*

Abstract: The reactions of iodine and methanol with a number of differently substituted derivatives of 1-(5-deoxy-β-D-*erythro*-pent-4-enofuranosyl)uracil have been examined. The 2′,3′-di-*O*-acetyl or di-*O*-benzoyl derivatives lead to 5′-deoxy-5′-iodo-4′-methoxy nucleosides with both the β-D-ribo and the α-L-lyxo configurations as well as to diastereomeric 4′,5′-ortho esters. On the other hand, the olefins containing 2′,3′-diol or 2′,3′-cyclic carbonate functions lead stereoselectively to 5′-iodo-4′-methoxy adducts with only the β-D-ribo configuration. Both β-D-ribo and α-L-lyxo adducts arise from the corresponding 2′,3′-*O*-isopropylidene derivative. The various products have been correlated by chemical interconversions and free 4′-methoxyuridine has been obtained via displacement of the 5′-iodo group in a suitable derivative by benzoate anion followed by hydrolysis to the free alcohol function. Acid-catalyzed equilibration of the 4′-methoxy nucleosides has been demonstrated and considerable data on the proton and ^{13}C NMR spectra of the various products are presented.

Recent years have witnessed the synthesis of a vast number of nucleoside analogs in the search for therapeutically useful agents.[1] These have included an impressive variety of base analogs of the normal purine and pyrimidine nucleosides as well as a myriad of variations of the sugar moiety. With respect to the latter, extensive modification of the stereochemistry and functionalization of most of the carbon atoms in the normal ribose or 2-deoxyribose unit has been achieved. Due largely to a paucity of convenient synthetic methods for introducing functionality at C_4 of a furanose sugar, only very few modifications at $C_{4'}$ of nucleosides have been reported. These have included the introduction of either 3′,4′[2] or 4′,5′[3] unsaturation, replacement of the furanose ring oxygen by sulfur,[4] and inversion of configuration leading to α-L-lyxofuranosyl nucleosides.[2b,5]

Impetus for the development of synthetic methodology leading to the preparation of $C_{4'}$-substituted nucleosides came from the elucidation of the structure of the nucleoside antibiotic nucleocidin as 4′-fluoro-5′-*O*-sulfamoyladenosine.[6] This structure was unique in that it is the only naturally occurring fluoro sugar derivative and also the first reported 4-substituted glycofuranoside. We have initiated a broad program concerning the synthesis of variously 4′-substituted nucleosides and have previously described a synthesis of nucleocidin.[7] A part of our work has recently been surveyed.[8] During the course of our work the synthesis of a novel methyl 4-methoxyhexofuranoside was described by Dmytraczenko et al.[9] via an interesting concerted addition-elimination reaction on a 5-keto-6-tosylhexofuranoside. Also, the preparation of a 4-alkoxy-5-deoxy-β-D-ribofura-